Antibodies to Pancreatic Islet Cell Antigens in Diabetes Seen in Southern India with Particular Reference to Fibrocalculous Pancreatic Diabetes

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Fibrocalculous pancreatic diabetes (FCPD) is a type of diabetes secondary to tropical chronic non-alcoholic pancreatitis. Little is known about the aetiopathogenesis of FCPD. We studied glutamic acid decarboxylase antibodies (GAD-Ab) and islet cell antibodies (ICA) in patients with FCPD and compared the results with Type 1 (insulin dependent) diabetes mellitus, Type 2 (non-insulin-dependent) diabetes mellitus and non-diabetic subjects in Southern India. The prevalence of GAD-Ab was 7.0 % (95 % Confidence Interval (CI) 1.9–17.2) in FCPD, 47.5 % (CI 31.4–64.0) in Type 1 ($p < 0.001$ compared to FCPD), 5.6 % (CI 1.5–13.9) in Type 2 (non-significant (NS) compared to FCPD) and 0 % in controls. The prevalence of ICA was 6.3 % (CI 1.2–17.4) in FCPD, 53.8 % (CI 37.1–70.0) in Type 1 ($p < 0.001$ compared to FCPD), 9.9 % (CI 4.0–19.4) in Type 2 (NS compared to FCPD) and 4.7 % (CI 0.4–16.1) in controls. The data suggest that in FCPD, the frequency of auto-antibodies is low and its aetiology is probably not linked to autoimmunity in the majority of the patients. © **1998 John Wiley & Sons, Ltd.**

Diabet. Med. **15: 156–159 (1998)**

KEY WORDS **fibrocalculous pancreatic diabetes; Type 1 diabetes mellitus; Type 2 diabetes mellitus; GAD antibodies; islet cell antibodies; Southern India**

Received 7 March 1997; revised 21 August 1997; accepted 29 August 1997

Introduction

Among South Indian subjects, in addition to Type 1 and Type 2 diabetes mellitus (the two major forms of diabetes seen among Europeans), there is another class of diabetes known as fibrocalculous pancreatic diabetes (FCPD). This is a form of diabetes secondary to tropical non-alcoholic chronic pancreatitis.[1,2] Southern India has the highest known prevalence of FCPD in the world, with a frequency of 1:1000 reported in a population-based survey.[3] FCPD is characterized by severe diabetes with onset in youth but with an absence of ketonuria on withdrawal of insulin and, additionally, evidence of chronic pancreatitis.[4] Pancreatic auto-antibodies have been reported in an isolated case of FCPD.[5] A recent study has looked at islet cell antibodies (ICA) in FCPD seen in Northern India.[6] However there are no reports on glutamic acid decarboxylase (GAD-Ab) antibodies in FCPD or indeed any of the other forms of diabetes seen in Southern India.

In this paper we report on the distribution of GAD-Ab and ICA in patients with various types of diabetes and particularly FCPD in a South Indian diabetes clinic population.

Patients and Methods

Subjects

The study was conducted on 57 patients with FCPD, 40 patients with Type 1 and 71 with Type 2 diabetes mellitus, all attendees of the M.V. Diabetes Specialities Centre at (Chennai) Madras, Southern India, as well as in 45 healthy non-diabetic individuals. All investigations in patients were carried out at the time of first presentation to the centre. Clinical details are shown in Table 1. The diagnosis and classification of the three types of diabetes were based on standard criteria.

FCPD was diagnosed as described.[4,7] All patients

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 CCC 0742–3071/98/020156–04$17.50

Table 1. Clinical details of study groups

	Controls ($n = 45$)	Type 1 ($n = 40$)	FCPD ($n = 57$)	Type 2 ($n = 71$)
Male : female	18 : 27	23 : 17	39 : 18	48 : 23
Age (yr)	29 ± 8[a]	20 ± 12[a]	37 ± 12[a]	48 ± 11[a]
Body mass index (kg m^{-2})	21.9 ± 3.0	17.8 ± 3.8	19.7 ± 3.0	25.2 ± 3.8
Fasting plasma glucose (mg dl^{-1})	86 ± 9	234 ± 112	167 ± 107	168 ± 61
Glycosylatd haemoglobin (%)	5.9 ± 0.7	12.9 ± 2.8	8.7 ± 2.8	9.5 ± 2.8
Fasting C-peptide (pmol ml^{-1})	0.64 ± 0.27[a]	0.09 ± 0.10[a]	0.24 ± 0.16[a]	0.46 ± 0.23[a]
Postprandial C-peptide (pmol ml^{-1})	1.85 ± 0.56[a]	0.14 ± 0.08[a]	0.53 ± 0.30[a]	1.20 ± 0.50[a]
Mean duration of diabetes (yr)	–	2.0 ± 1.7	7.0 ± 5.1	0.3 ± 0.2
GAD-Ab positivity, number (%) (95 % CI)	0/45 (0 %)	19/40 (47.5) (31.4–64)	4/57 (7 %)[c] (1.9–17.2)	4/71 (5.6 %)[c] (1.5–13.9)
ICA positivity, number (%) (95 % CI)	2/43 (4.7 %) (0.4–16.1)	21/39 (53.8) (37.1–70)	3/48 (6.3 %)[c] (37.1–70)	7/71 (9.9 %)[d] (4.0–19.4)
GAD-Ab (or) ICA positivity, number (%) (95 % CI)	2/43 (4.7 %) (0.4–16.1)	23/39 (59 %) (42.0–74.5)	7/48 (14.6 %)[d] (6.0–27.9)	10/71 (14.1)[d] (6.9–24.5)

FCPD, fibrocalculous pancreatic diabetes mellitus; CI, confidence interval.
Data are mean + SD.
[a] $p < 0.001$ compared to all other groups.
[b] $p < 0.001$ compared to control and NIDDM.
[c] Not significantly different from zero.
[d] Significantly different from zero.

had evidence of diabetes, gave a history of recurrent abdominal pain from childhood suggestive of pancreatitis, and had evidence of pancreatic calculi on plain abdominal X-ray and/or pancreatic ductal dilatation with intraductal stones on ultrasonography. No patient gave a history of alcohol intake or had hypercalcaemia or biliary tract disease. All patients with FCPD required insulin for control of diabetes and none gave a history of diabetic ketoacidosis or evidence of ketonuria.

Type 1 (insulin dependent) diabetes mellitus was diagnosed according to an abrupt onset of diabetes; requirement of insulin for control of hyperglycemia from the onset; susceptibility to ketosis in the basal state or documented episodes of ketoacidosis. None had a history suggestive of pancreatitis and all had normal abdominal X-rays and ultrasonograms.

Type 2 (non-insulin dependent) diabetes mellitus was diagnosed according to the WHO study group report criteria.[1] These patients responded to diet or diet and oral hypoglycaemic agents and were not prone to ketosis. None had a history suggestive of pancreatitis and all had normal abdominal X-rays and ultrasonograms.

The control group consisted of 45 healthy non-diabetic members of the staff of the M.V. Diabetes Specialities Centre in Madras with no family history of diabetes or autoimmune disease.

Laboratory Methods

Blood was drawn from all study subjects after an overnight fast and serum was stored at –20 °C until the determination of GAD-Ab and ICA assays which were done on coded sera. ICA were measured at Lucknow, India by indirect immunofluoresence.[6] An ICA titre of >5 JDF units was considered positive. The assay has been included in the 12th International Diabetes Workshop ICA Proficiency Program (1996). The sensitivity and specificity of the assay were 93 % and 88 %, respectively.

Sera for GAD-Ab were transported to Melbourne in dry ice and tested by radio-immunoprecipitation (RIP) assay as previously described.[8] A normal upper limit for GAD-Ab antibodies using the RIP assay of 18 units has been established both in healthy Caucasian[9] and Asian subjects.[10] The performance of this assay including intra- and inter-assay coefficients of variation (CV) has been described and validated at the first (France) and second (Italy) GAD antibody workshops.

Plasma glucose was estimated using glucose oxidase kits (Boehringer Mannheim, Mannheim, Germany) on an Opera Random Access Autoanalyser (Bayer Diagnostics, Tarrytown, New York, USA). Glycosylated haemoglobin (HbA_{1c}) was estimated by the high pressure liquid chromatography (HPLC) method using the Variant Machine (Bio Rad, USA). Plasma was separated and stored at –20 °C for C-peptide estimations which were done at Madras by Elisa using the Dako kit (Dako Diagnostics Ltd, Ely, UK). C-peptide determinations were done in the fasting state and after stimulation by a standard breakfast as previously described.[11] The intra-assay and inter-assay coefficient of variation for C-peptide assays were 4.0 % and 8.3 %, respectively, and the lower detection limit was 0.02 pmol ml^{-1}. Informed consent was obtained from all study subjects and the study was approved by the institutional ethics committee.

Statistics

Data are expressed as mean ± standard deviation (SD). Continous variables were analysed using one-way ANOVA and unpaired students *t*-test and categorical

variables were analysed by the chi-square test with Yates correction or Fisher's exact test using two-tailed p values; p values < 0.05 were considered significant.

Results

The patients with FCPD were intermediate in age between those with Type 1 and those with Type 2 disease, and these differences were highly significant, $p < 0.001$ (Table 1). The BMI of FCPD and Type 1 patients were lower compared to the Type 2 patients and controls ($p < 0.001$). Fasting plasma glucose levels were higher in Type 1 compared to FCPD ($p = 0.02$) and Type 2 ($p = 0.006$). HbA_{1c} levels were also higher in IDDM ($p < 0.001$) There were no significant differences in fasting plasma glucose and HbA_{1c} between FCPD and Type 2 patients. Fasting and postprandial C-peptide levels in FCPD were intermediate between Type 1 and Type 2 groups, confirming our earlier studies.[12]

GAD-Ab was present in 47.5 % (19/40) of Type 1 patients, 7.0 % (4/57) of patients with FCPD, 5.6 % (4/71) of patients with Type 2 and in none of the controls. ICA was measured in most subjects. ICA positivity was seen in 53.8 % (21/39) of the Type 1 patients, 6.3 % (3/48) of FCPD patients, 9.9 % (7/71) of Type 2 patients and 4.7 % (2/43) of the controls. GAD-Ab or ICA positivity was seen in 4.7 % of controls, 59 % of Type 1 patients, 14.6 % of FCPD patients, and 14.1 % of Type 2 patients. The confidence intervals are also shown in Table 1.

Figures 1(a) and 1(b) show the GAD-Ab and ICA positivity in relation to duration of diabetes. We tested 22 and 21 sera of Type 1 patients with less than 1 year of disease for GAD-Ab and ICA, respectively, and 7 and 6 of the sera from FCPD patients. Both GAD-Ab and ICA positivity were higher in Type 1 patients with shorter duration of diabetes. Among FCPD with over 5 years duration, 10.3 % were GAD-Ab positive and 9.1 % were ICA positive.

There were no significant difference between the GAD-Ab or ICA positive and negative cases with respect to age, sex, body mass index, plasma glucose, glycosylated haemoglobin or C-peptide levels between the three patient groups. There was also no relationship between ICA or GAD-Ab positivity and occurrenceof ketoacidosis among the Type 1 patients. Multiple logistic regression analysis showed no association between antibody positivity and age, sex, duration, BMI, plasma glucose, and C-peptide levels in any of the three study groups.

Discussion

This paper presents data on GAD-Ab and ICA in three forms of diabetes seen in Southern India. The antibody positivity and their titres are highest among the Type 1 diabetic patients, especially those with short duration. Our figures are not dissimilar to those reported among Europeans.[13–15] Among patients with Type 2 diabetes, the small number of antibody positive patients may represent a slowly progressive form of Type 1 disease, previously known as Latent Autoimmune Diabetes of Adults (LADA).[16]

Among FCPD, the number of patients with less than 1 year duration was small, but none had ICA and only 1 of 7 was GAD-Ab positive—less than would be seen in frankly autoimmune Type 1 disease. Around 10 % FCPD patients with longer duration of diabetes were GAD-Ab and/or ICA positive and these may represent patients diagnosed to have FCPD who also have a slowly progressive form of Type 1 disease. We have earlier shown that FCPD shares susceptibility genes in common with both Type 1 and Type 2 diabetes.[17] More studies are clearly needed to clarify this aspect further.

In conclusion, studies on pancreatic islet cell antibodies in diabetes in Southern India show that Type 1 patients have GAD-Ab and ICA frequencies that are similar to those of Europeans while their frequency in Type 2 disease and in FCPD is low. The antibody positivity in FCPD and Type 2 disease could be attributed to misclassification. Further studies on HLA and other markers are needed on these patients.

Acknowledgements

We thank C.S. Shanthi Rani, K. Karkuzhali, G. Premalatha, and L. Varkey of the M.V. Diabetes Specialities Centre,

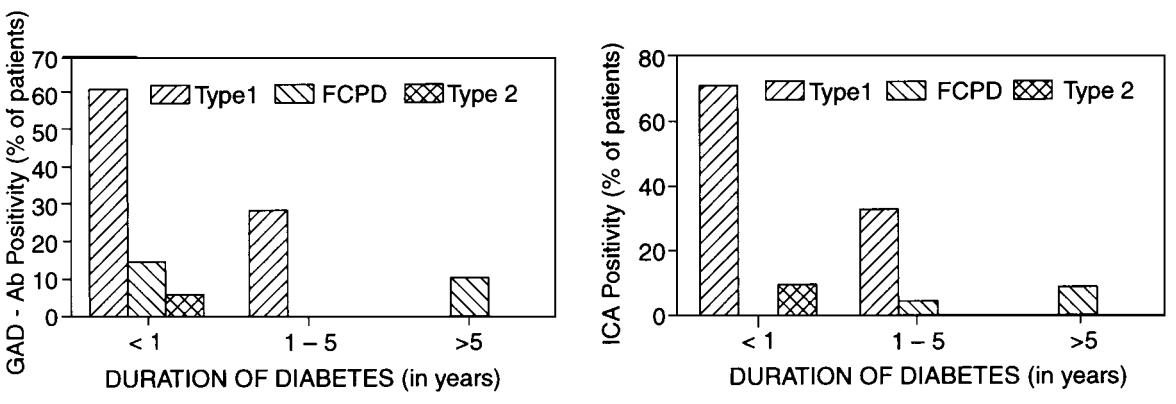

Figure 1. (a) GAD-Ab positivity and (b) ICA positivity in relation to duration of diabetes in the study groups

Madras for their help in this study. We are also grateful to A.K. Mathai for the statistical analysis and to R. Spark for technical help with the GAD-Ab assay.

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